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Increased von Willebrand Factor Binding to Platelets in Single Episode and Recurrent Types of Thrombotic Thrombocytopenic Purpura

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Extensive microvascular platelet aggregation is characteristic of thrombotic thrombocytopenic purpura (TTP). Previous studies have indicated that abnormalities of von Willebrand factor (vWf) are often present in TTP patient plasma. There has not been previously any direct evidence linking these abnormalities to the process of intravascular platelet aggregation in TTP. We used flow cytometry to analyze the binding of vWf to single platelets, and the presence of platelet aggregates, in the blood of 4 children with chronic relapsing (CR) TTP and 5 adults with single episode or recurrent TTP. vWf on the single platelets of CRTTP patients at all time points studied was significantly increased compared to controls, and was increased further as platelet counts decreased to levels below 40,000/μl. The single episode and recurrent adult TTP patients had platelet aggregates in the blood, as well as increased vWf on single platelets, before therapy commenced and thereafter until recovery was in process. In the one unresponsive single episode TTP patient, vWf on single platelets remained elevated, and platelet aggregates persisted, until her death. The platelet α-granular protein, P-selectin, was not increased on the single platelets of most TTP blood samples, suggesting that it is vWf from plasma (rather than from α-granules) that attaches to platelet surfaces in association with platelet aggregation. These results suggest that vWf-platelet interactions are involved in the platelet clumping process that characterizes TTP. Am. J. Hematol. 57:293–302, 1998.

Key words: thrombotic thrombocytopenic purpura (TTP); chronic relapsing thrombotic thrombocytopenic purpura (CRTTP); platelets; von Willebrand factor (vWf); flow cytometry

INTRODUCTION

The pathological and clinical findings in TTP suggest that the process involves direct, potentially reversible, platelet aggregation in high-shear regions of the microcirculation of multiple organs concurrently [1–4]. Shear-induced platelet aggregation requires large or unusually large vWf multimers [5,6] that are produced and stored in endothelial cells [7]. Unusually large vWf multimers are more effective than the largest plasma vWf forms at binding under the influence of fluid shear stress to the GPIb-IX-V receptors and to GPIIb-IIIa complexes, and inducing aggregation [5,6]. The evaluation of serial plasma samples from patients during TTP episodes often demonstrates either the presence of unusually large vWf multimers or, alternatively, absence of the largest plasma vWf forms [8–12]. Immunohistochemical studies of TTP thrombi reveal an abundance of vWf with little fibrinogen/fibrin (the opposite findings are characteristic of thrombotic lesions in disseminated intravascular coagu-

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lation) [13–15]. The presence of unusually large vWf multimers in plasma is characteristic of chronic relapsing (CR) TTP patients both between and, to a lesser extent, during TTP episodes [8,9,16]. This report provides the first systematic analysis known to us of the association of vWf with single platelets, and the associated presence of platelet aggregates, in whole blood during episodes of different types of TTP.

MATERIALS AND METHODS

CRTTP Patients

The 4 CRTTP patients are children between the ages of 3 and 12 years who were diagnosed with CRTTP in infancy or early childhood because of severe thrombocytopenia and intravascular hemolysis (± neurological dysfunction), presence of unusually large vWf multimers in their plasma, rapid responsiveness to plasma infusion, and recurrence of the disorder about every 21 days [4,17]. Each of the patients presently receives prophylactic plasma infusion (10 ml/kg) every 3 weeks. Each CRTTP patient presents, therefore, a controllable opportunity for the regular study of many incipient or overt TTP relapses. In this study, the 4 CRTTP children were studied on 29 separate occasions immediately prior to their regular (every 3 weeks) prophylactic plasma infusions; on 9 of these occasions, the pre-infusion platelet counts had dropped below 40,000/μl.

Single and Recurrent TTP Patients

The 5 adult patients with acute episodes of TTP studied serially include 4 females and 1 male between the ages of 36 and 58 years. All had profound thrombocytopenia, microangiopathic hemolytic anemia, and extreme lactic acid dehydrogenase elevations at presentation, along with either central nervous system dysfunction (4 patients) or abdominal pain (1 patient). No patient had disseminated intravascular coagulation, heparin-associated thrombocytopenia/thrombosis, or antibodies to human immunodeficiency virus-1. Two patients (TS and RM) previously had TTP episodes within the preceding 4 years. One patient (JP) with an initial TTP episode had been treated with ticlopidine for 2 months following a myocardial infarction. All patients received high-dose glucocorticoids, and all were exchanged daily with fresh-frozen plasma or cryoprecipitate-poor plasma (cryosupernatant). Two patients (HJ and SS) were splenectomized during their initial episode of TTP. The 5 patients were studied serially (96 separate analyses) until recovery (4 patients) or death (1 patient).

This study was approved by the Institutional Reviews Boards of the Baylor College of Medicine and the Baylor Affiliated Hospitals. Adult patients, parents on behalf of their minor children, and healthy donors signed informed consent statements before blood samples were obtained. TTP patient blood samples were collected into separate 4-ml tubes containing a final concentration of either 3.7 mM potassium ethylene-diamine tetraacetate (EDTA) or 0.38% sodium citrate immediately before plasma infusion or exchange procedures.

Preparation of Anti-vWf

vWf was purified from normal human cryoprecipitate by glycine and NaCl fractionation [18], followed by agarose 4B column chromatography. The purified vWf forms used in this study were enriched in the largest multimers found in plasma and were capable of inducing the agglutination of washed platelets in the presence of 1.5 mg/ml ristocetin (Helena Laboratories, Beaumont, TX). Rabbits were immunized with the purified human vWf in Freund's Complete Adjuvant, and γ-globulin fractions of rabbit sera were prepared by repeated cycles of 33% ammonium sulfate precipitation. The polyclonal rabbit anti-vWf was monospecific, i.e., it identified only vWf antigen in normal plasma, and failed to react with the plasma of a severe von Willebrand's disease patient [5,6]. This anti-vWf is especially effective: in reporting the presence of unusually large vWf multimeric forms; and in detecting the binding of small amounts of the largest plasma vWf multimers or unusually large vWf forms to single platelets during the initiation of platelet aggregation induced by shear stresses above about 60 dynes/cm^2 [5,6,19–21]. At these elevated shear stresses, vWf is the only ligand that binds to the platelet receptors glycoprotein (GP) Ib-IX-V and GPIIb-IIIa [5,6,19].

Analysis of Platelet-vWf Binding

Aliquots (5 μl) of EDTA-whole blood were incubated with saturating concentrations of the rabbit polyclonal anti-human vWf IgG specific for human vWf (anti-vWf; 2.5 μg/ml) and FITC-conjugated polyclonal goat anti-rabbit IgG (anti-IgG-FITC; 5 μg/ml; Vector Laboratories, Burlingame, CA) in 0.22 μM-filtered 10 mM HEPES/BSA, pH 7.4, for 30 min in the dark at room temperature. The samples were fixed with 1% formaldehyde, diluted with filtered phosphate-buffered-saline (PBS), pH 7.4, and analyzed for mean fluorescence in a flow cytometer (FACScan, Becton Dickinson, San Jose, CA).

Light scatter and fluorescence channels were set at logarithmic gain. Platelets were distinguished from erythrocytes, leukocytes, and small particulate debris on the basis of characteristic platelet forward- and side-scatter profiles [22] (Fig. 1A) and confirmed by reactivity with the mouse monoclonal antibody, anti-CD42a-FITC (Becton Dickinson) that identifies the GP IX portion of the GPIb-IX-V complex. A gate was set around single platelets, and 10,000 events were acquired. FITC fluorescence of the gated single platelets was then analyzed as an index of binding of the reporter anti-vWf and anti-IgG-FITC to platelet surfaces. FITC fluorescence of

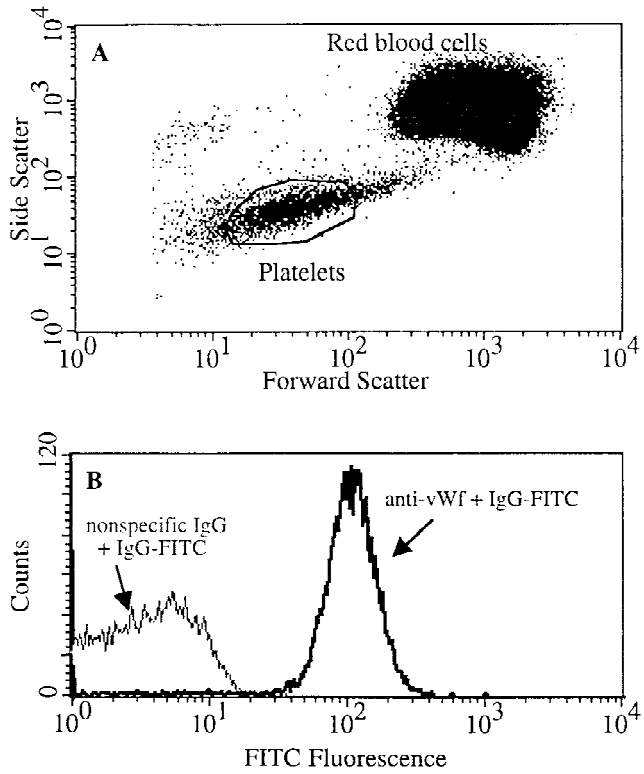

Fig. 1. A: Flow cytometry identification of platelets in whole blood. Platelets were differentiated from red blood cells on the basis of their characteristic forward- and side-scatter pattern. A gate was set around single platelets and 10,000 events were acquired. B: Botrocetin-induced vWf binding to platelets in whole blood. Aliquots (5 µl) of unstirred normal EDTA-whole blood samples in the presence of 4 µg/ml botrocetin were incubated for 30 min with an irrelevant rabbit anti-rat IgG and goat anti-rabbit IgG-FITC (nonspecific IgG + IgG-FITC) or rabbit anti-human vWf IgG and goat anti-rabbit IgG-FITC (anti-vWf + IgG-FITC). This was followed by fixation and FACScan analysis. vWf binding to platelets induced by botrocetin is expressed as a positive shift in the mean fluorescence from background values.

only single platelets was analyzed to ensure that any observed increase in fluorescence would not simply be the additive result of subthreshold vWf binding to multiple platelets in an aggregate. The binding results were expressed as mean fluorescence of vWf-positive single platelets minus background fluorescence. Background fluorescence was represented by values obtained: in the presence of goat anti-rabbit IgG-FITC (Vector Laboratories), but the absence of rabbit anti-human-vWf; or, alternatively, in the presence of a nonspecific rabbit anti-rat IgG (Vector Laboratories) and goat anti-rabbit IgG-FITC. vWf-positive platelets were produced by adding 4 µg/ml of the snake venom protein, botrocetin (Pentapharm, Basel, Switzerland), in order to induce intense vWf binding (Fig. 1B) [23]. Botrocetin binds specifically to vWf multimers of various sizes and induces binding of the botrocetin-vWf complexes to platelet GPIb components in GPIb-IX-V. More than a 10-fold positive shift in mean FITC fluorescence was observed in the presence of botrocetin, indicating specific botrocetin-vWf binding to platelets.

Analysis of Platelet P-Selectin

Platelet activation in 5-µl samples of citrate-whole blood was detected by flow cytometry using the reporter mouse monoclonal antibody, anti-CD62, labeled with phycoerythrin (anti-CD62-PE; 1 µg/ml, Becton Dickinson) [24,25]. Analysis was done within 1 h of sample acquisition. The antigen CD62 is the α-granule protein, P-selectin (GMP-140), that is secreted by activated platelets onto the platelet surface membrane [26]. A control irrelevant monoclonal antibody, gamma 1-PE (γ_1-PE; 2 µg/ml, mouse anti-keyhole limpet IgG_1, Becton Dickinson), was used to determine non-specific binding of a PE-labeled antibody. This non-specific fluorescence was subtracted from each sample.

Stimulation of Platelets With Ristocetin and ADP

One milliliter of citrate-whole blood samples from healthy donors was mixed gently by inversion (no stirring) for 1 min at room temperature with either no agonist, 20 µM ADP, or 1.2 mg/ml ristocetin. In the absence of stirring, no platelet aggregation or agglutination occurred. Aliquots of 100 µl were fixed with an equal volume of 2% formaldehyde in PBS. The samples were analyzed for mean fluorescence using anti-vWf and anti-P-selectin by flow cytometry.

Platelet Aggregates in the Plasma of TTP Patients

Platelet populations, using either EDTA- or citrate-whole blood, were selected by CD42a-FITC and analyzed using forward- and side-scatter flow cytometry profiles [25]. Single platelets (≤4.5 µm) were differentiated from aggregates based on the forward scatter light intensity values, which are size-related [27,28]. The presence of platelet aggregates in patient samples was indicated by platelet events larger than single platelets. Normal control samples ($n = 44$) contained few aggregates (Fig. 2A) and there was no significant difference between EDTA- and citrate-whole blood samples. In order to produce shear-induced platelet aggregates (Fig. 2B) in vitro as positive controls for the analysis of TTP patient samples, normal citrate-whole blood samples were subjected to 30–150 dynes/cm^2 shear stresses for 1 min at room temperature in a cone-and-plate viscometer [5,6,19]. The sheared normal blood was immediately fixed in an equal volume of 2% formaldehyde in PBS, incubated with anti-CD42a-FITC, diluted with 1% formaldehyde/PBS, and analyzed by flow cytometry. The extent of aggregation increased as the level of applied shear stress was raised (data not shown).

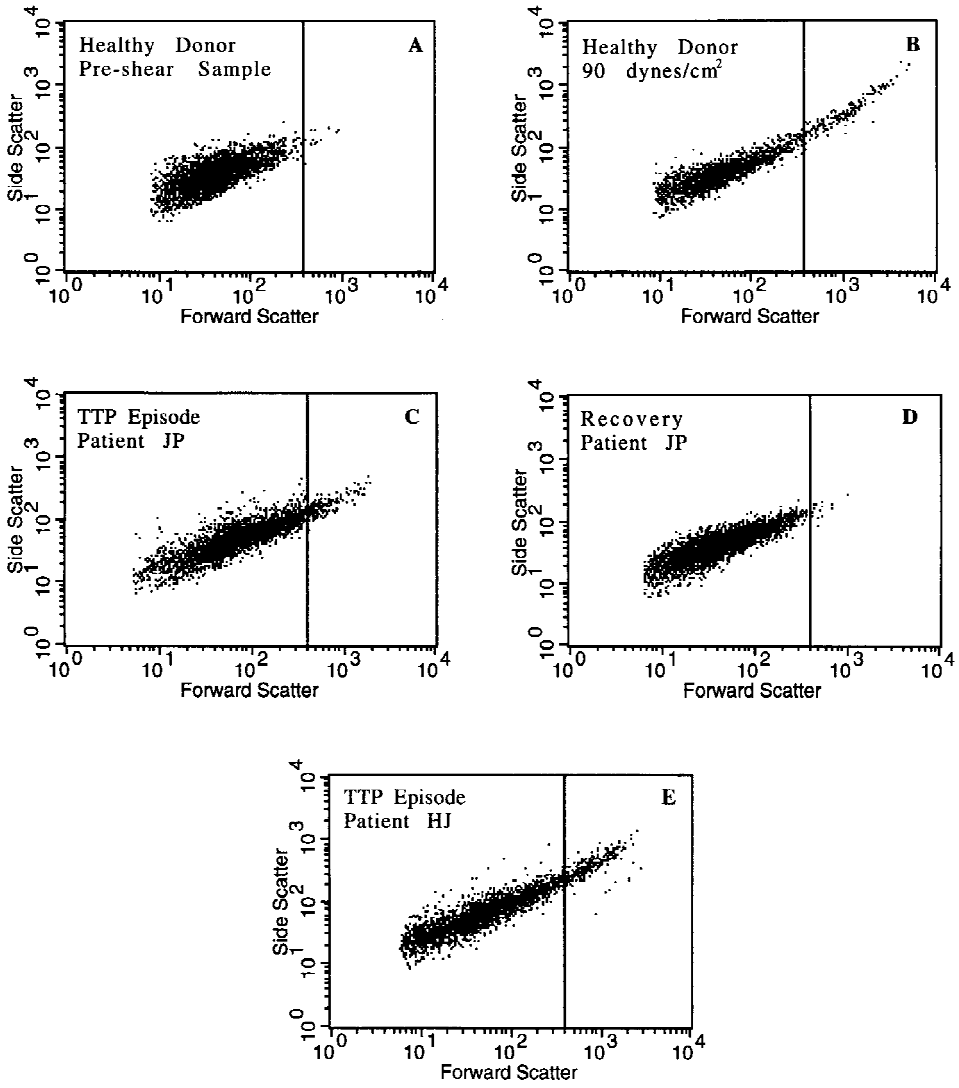

Fig. 2. Detection of platelet aggregates by flow cytometry. A representative normal citrate-whole blood sample before (A) and after (B) the application of 90 dynes/cm² shear stress in a cone-and-plate viscometer for 1 min at room temperature. Samples were fixed, incubated with anti-CD42a-FITC (anti-GPIX portion of the GPIb-IX-V complex), and analyzed by FACScan. Platelet populations were selected by FITC fluorescence and analyzed for size using forward-scatter light intensity. The vertical line separates single platelets from platelet aggregates. Aggregates detected in the blood of patient JP during her TTP episode (C) almost disappeared after her recovery (D). The near absence of platelet aggregates upon recovery was observed in the blood of all of the TTP patients except HJ. The platelet counts of HJ never rose above 20,000/ml, and platelet aggregates persisted until her death (E).

vWf Multimeric Patterns

EDTA-plasma samples from the TTP patients were analyzed for vWf multimeric patterns by sodium dodecyl sulfate (SDS)-1% agarose gel electrophoresis [5,6,8,9, 19,20,27], followed by electrical transfer to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). This membrane was then overlaid with polyclonal rabbit anti-vWf labeled with peroxide, and the vWf multimeric patterns were displayed with chemiluminescence [29].

Statistical Analysis

The data are expressed as means ± standard error of the mean. Statistical significance of differences between means was determined by paired Student's *t*-test, unless specified otherwise. Specifically, the reported *P* values for statistical comparison between TTP and normal values were determined by paired Student's *t*-test on TTP samples and corresponding normal control samples obtained on the same sampling dates.

RESULTS

Platelet-vWf in CRTTP Children

It has previously been demonstrated that each of the CRTTP patients has unusually large vWf forms in their EDTA plasma between incipient TTP relapses. The relative intensity of unusually large vWf often decreased by SDS-1% agarose gel analysis as relapses began and platelet counts dropped [4,17]. Venous whole blood samples from 4 CRTTP patients studied immediately prior to their every 3 week prophylactic plasma infusions had more vWf binding than platelets in normal control blood studied at the same time ($P = 0.003$; Table I). This increase in vWf on single platelets was observed in samples with platelet counts either above or below 40,000/μl. Furthermore, the extent of vWf binding to single platelets in CRTTP blood samples with platelet counts <40,000/μl ($n = 9$) was significantly greater than that in CRTTP blood samples with platelet counts >40,000/μl ($n = 20$; $P = 0.015$, unpaired Student's *t*-

TABLE I. vWf Binding to Single Platelets in the EDTA Blood of TTP Patients

	Platelet count (per μl)	Mean platelet-vWf fluorescence (arbitrary units)
Controls	>200,000	4.4 ± 0.3
Chronic relapsing TTP	<40,000	9.9 ± 1.3*
Chronic relapsing TTP	>40,000	6.0 ± 0.6*
Single and recurrent TTP episodes		
Initial 2 days	<40,000	12.6 ± 1.8*
Recovery	>200,000	4.9 ± 0.8

*Indicates significantly different from controls.

test). These data indicate that, even between episodes, the extent of vWf binding to the single platelets in CRTTP patient blood is greater than in normal control blood. On those occasions when platelet counts decreased below 40,000/μl just before a scheduled prophylactic plasma infusion in the CRTTP children, further increase in vWf was detected on the single platelets of the patients.

Platelet-vWf in Single Episode and Recurrent Adult TTP Patients

These 5 patients, who were studied serially during TTP episodes, all had significantly elevated levels of vWf on single platelets from that of normal controls ($P = 0.001$, Table I) during the initial 2 days of hospitalization when their platelet counts were lowest (ranging from 5000/μl to 40,000/μl). As platelet counts increased with glucocorticoid/plasma exchange therapy into or above the normal range (206,000/μl to 562,000/μl) in 4 of the patients, vWf on single platelets concurrently decreased toward or into the normal range.

If a transient decrease in platelet numbers recurred during therapy, as in patient TS on days 20 and 21 (Fig. 3), then vWf on single platelets again increased transiently. The serial studies of the other adult TTP patients are shown in Figures 4 to 7. In two of the patients, TTP episodes were recurrent (Figs. 3 and 4). Three patients were studied during an initial episode of TTP (Figs. 5–7). TTP was associated with exposure to ticlopidine in one of these patients (Fig. 5). Platelet counts in the one unresponsive patient (HJ) never rose above 20,000/μl, and the increased vWf on her single platelets persisted until death (Fig. 7).

Platelet-vWf in Normal Controls and Other Patients

vWf on single platelets was not increased above normal in 13 EDTA whole blood samples from 11 children (ages 3 to 17 years) who had platelet counts between 1,000 and 148,000/μl) because of defective production or excessive destruction of platelets (by disorders other than TTP). Six of these children had platelet counts below 40,000/μl. There was no increase above normal in vWf on single platelets in 2 adult patients who had disseminated intravascular coagulation and 2 adult patients who had idiopathic (autoimmune) thrombocytopenia (range of platelet counts from 30,000/μl to 72,000/μl). The absence of increased platelet-vWf values in these non-TTP patients indicates that the presence in blood of larger, presumably young platelets with increased surface areas does not explain the increased singlet platelet-vWf values in the TTP patients. Furthermore, serial dilution of EDTA-whole blood obtained from healthy donors to produce platelet counts of 10,000/μl did not result in any significant increase in vWf on single platelets compared to undiluted normal samples (data not shown). These results indicate that thrombocytopenia alone in the presence of constant concentrations of anti-vWf and anti-IgG-FITC antibodies does not lead artifactually to increased vWf mean fluorescence values on single platelets.

P-Selectin

P-selectin-PE fluorescence values on single platelets from 39 healthy donors obtained during the study of 3 of the adult TTP patients was 3.50 ± 0.57. Elevated levels of P-selectin were detected on the single platelets of only 2 TTP blood samples obtained on 46 different days during the serial analyses (Figs. 5 and 6). Of these 46 samples, 23 had elevated levels of vWf on single platelets. The disparate vWf/P-selectin results indicate that the increased vWf on single platelets is not likely to be the result of platelet activation and the secretion of vWf from platelet α-granular onto the platelet surface, but rather the binding to single platelets of exogenous vWf from plasma.

Results of the platelet-vWf/P-selectin studies in the 3 TTP patients were qualitatively similar to results obtained in vitro by stimulating platelets in normal unstirred citrate-whole blood by 1.2 mg/ml ristocetin (which induces the specific binding of vWf to GPIb-IX-V complexes on platelets). vWf on platelets increased from 3.1 ± 0.5 (no agonist) to 81.6 ± 19 in the presence of ristocetin ($P = 0.001$), with no significant increase in P-selectin-PE fluorescence (0.8 ± 0.8 for no agonist and 1.90 ± 1.6 following addition of ristocetin). In contrast, stimulation of platelets in normal control citrate-whole blood by 20 μM ADP caused an increase in P-selectin to 22.2 ± 4.9 ($P = 0.001$) with no significant change in platelet-bound vWf (3.1 ± 0.5 for no agonist and 4.8 ± 0.8 for ADP samples).

Correlation of Platelet-vWf With Plasma vWf Multimeric Pattern

Concurrent study of vWf multimeric patterns in the EDTA-platelet-poor plasma of the 5 adult patients (Figs. 3–7) demonstrated that during the protracted TTP episodes: unusually large vWf forms were detected in 53/96

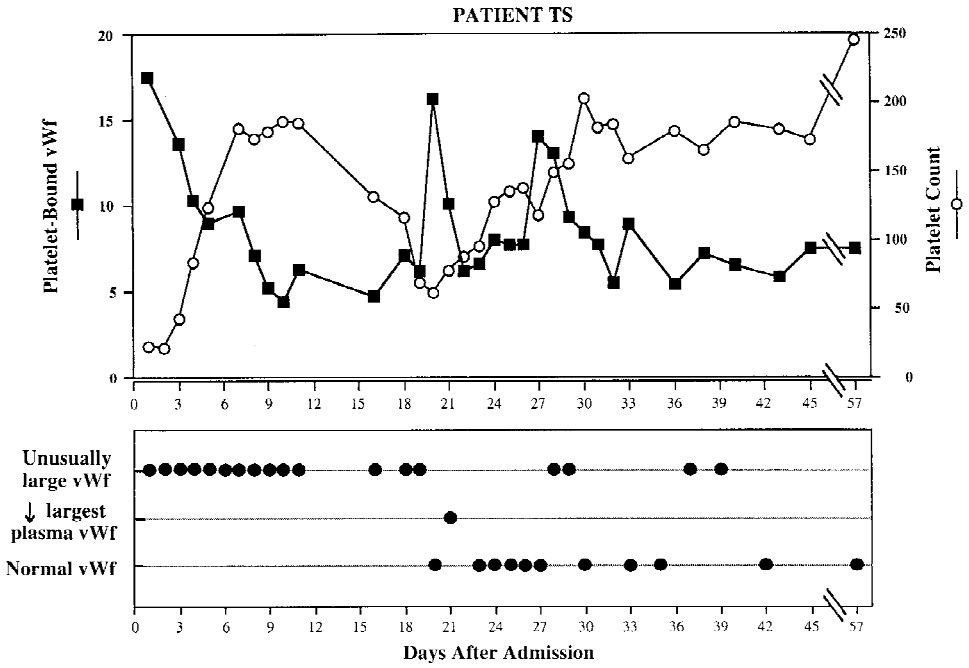

Fig. 3. Platelet counts, vWf on single platelets in EDTA-blood, and vWf multimeric patterns in EDTA-plasma in patient TS during his second TTP episode within 2 years. Plasma exchange with fresh-frozen plasma (FFP) or cryo-supernatant was performed daily for an additional 5 days after the platelet count exceeded 200,000/μl. No patient citrate-blood sample contained platelets that exceeded control (healthy donor) values for P-selectin expression. Samples were analyzed for P-selectin on 24 different days.

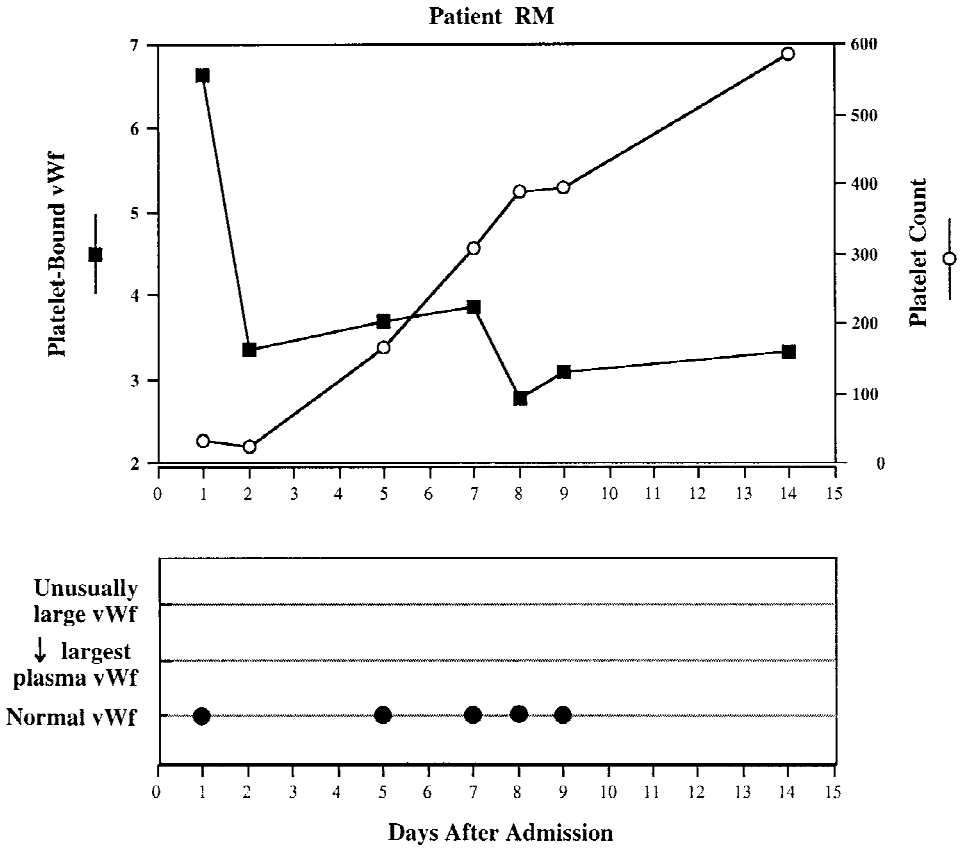

Fig. 4. Platelet counts, vWf on single platelets, and vWf multimeric patterns in patient RM during her fifth TTP episode within 4 years.

samples (55%); disappearance of the large plasma vWf multimers was found in 8/96 (8%); and normal vWf multimers were present in 35/96 samples (36%). Abnormal vWf multimeric patterns were, therefore, found in 61/96 plasma samples (64%). vWf multimeric patterns on plasma samples obtained when the adult patient platelet counts were below 150,000/μl, were as follows: unusually large vWf forms were present in 42/69 samples (61%); disappearance of the large plasma vWf multimers in 8/69 samples (12%); and normal vWf multimers in

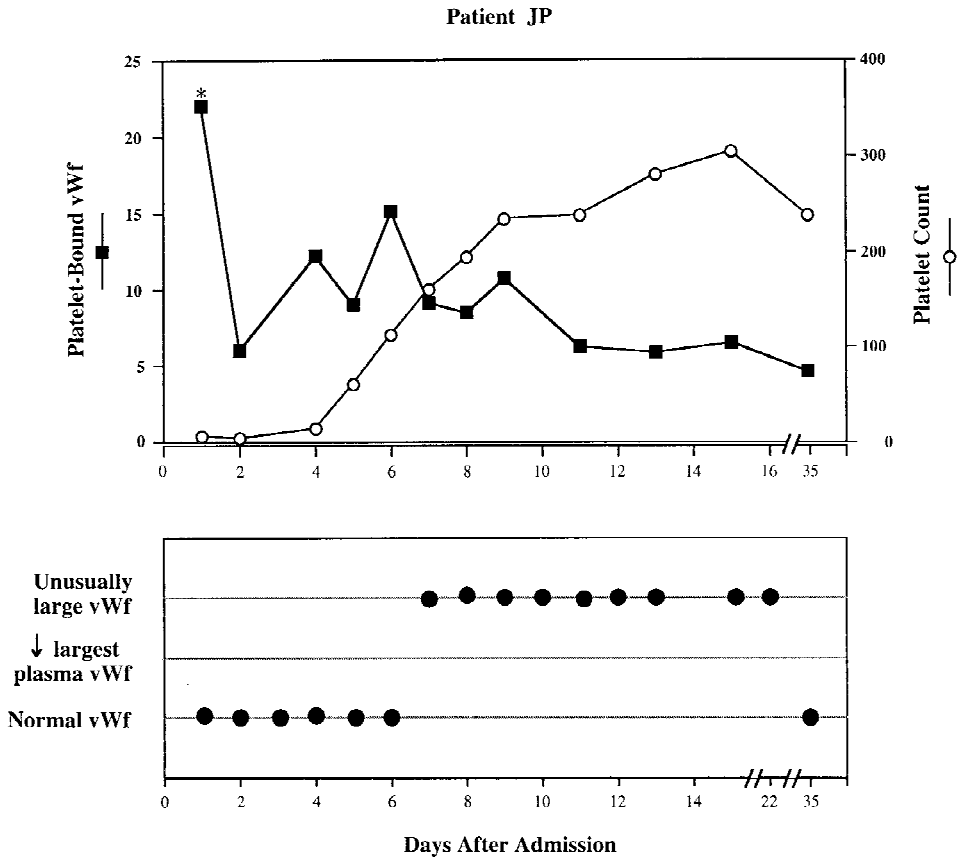

Fig. 5. Platelet counts, vWf on single platelets, and vWf multimeric patterns in patient JP, who had received ticlopidine prior to her single episode of TTP. The first of 7 citrated-blood samples (indicated by *) obtained on 7 different days contained platelets that exceeded control values for P-selectin expression.

19/69 samples (27%). Abnormal vWf multimeric patterns were found in 50/69 of this subgroup of plasma samples (73%). The flow cytometry data indicate that vWf on single platelets was inevitably increased in single episode and recurrent TTP patients, especially at the outset of the disorder (Table I).

One peculiar observation was the appearance of unusually large vWf forms in serial samples of JP, the ticlopidine-associated TTP patient, as she began to recover from her TTP episode (Fig. 5). During this recovery phase, the vWf on her single platelets decreased. This combination of observations suggests that mild endothelial cell perturbation, with release of unusually large vWf forms below a threshold rate required for binding to patient platelets, may have continued even as remission commenced.

Platelet Aggregates Detected Ex Vivo

At the onset of her TTP episode, the citrate-blood from patient JP contained many platelets in the size range of aggregates (Fig. 2C). After 2 weeks of plasma-exchange, aggregation in JP blood samples (Fig. 2D) returned to a level within the normal range for healthy donors ($n = 44$). The extent of platelet aggregation observed in the blood of TTP patients was similar to that produced in vitro by an abnormally elevated level of shear stress (Fig. 2A and B). Serial studies of the blood of the 5 adult TTP patients indicate that early during each TTP episode, lower platelet counts were associated with an increased extent of platelet aggregation. This abnormally increased aggregation in blood samples of 4 of the adult patients returned to within the normal range upon recovery (Fig. 2C and D). In the case of unresponsive patient HJ, the extent of platelet aggregation remained abnormally high until her death, and her platelet count never rose above 20,000/μl (Fig. 2E).

DISCUSSION

The extent of vWf binding to TTP patient single platelets was significantly increased during relapses relative to remission periods in the patients with single episode and recurrent types of TTP. It should be noted, however, that our flow cytometry studies cannot distinguish the size of vWf multimers bound to platelets (e.g., unusually large vWf forms vs. the largest vWf multimers in plasma). Furthermore, although our analyses can estimate the extent of binding of vWf to single platelet populations, we cannot quantify precisely the number of vWf multimers/platelet.

The data we obtained were from patient whole venous blood samples, and only single platelets were evaluated

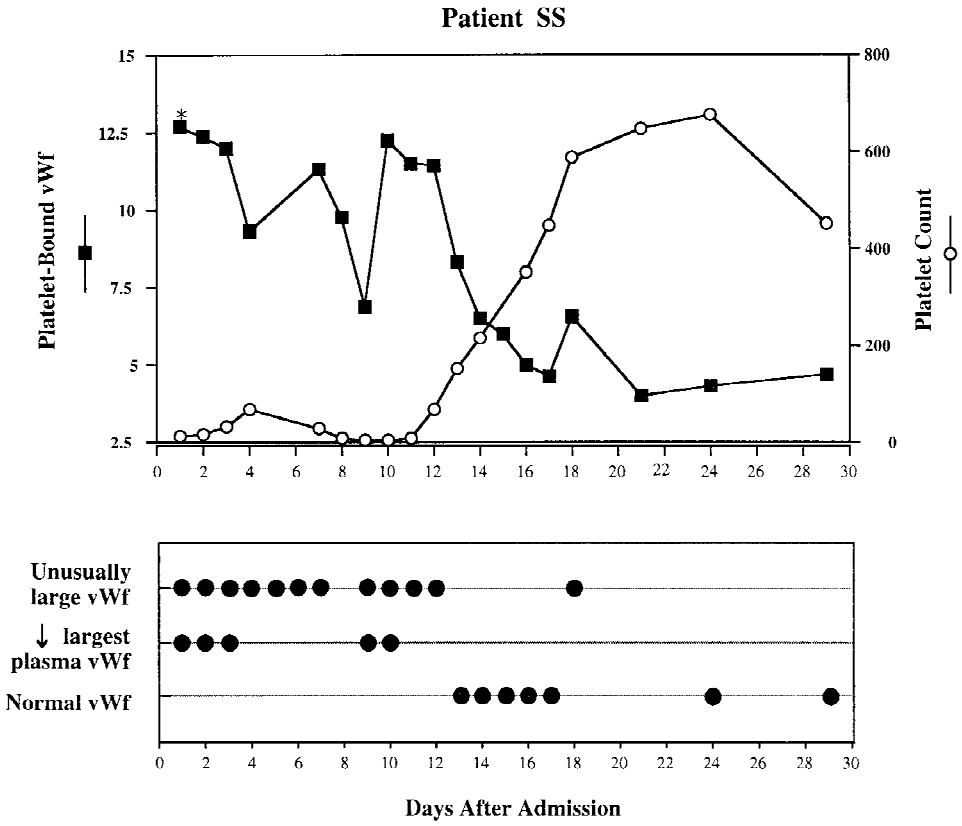

Fig. 6. Platelet counts, vWf on single platelets, and vWf multimeric patterns in patient SS, during her one idiopathic TTP episode. On several days, her plasma samples contained unusually large vWf forms and also lacked the largest plasma vWf multimers that are present in normal plasma. Splenectomy was performed on day 9. The first of 15 citrate-blood samples (indicated by *) obtained on 15 different days contained platelets that exceeded control values for P-selectin expression.

for vWf-platelet interactions. TTP is predominantly associated with reversible platelet aggregation in arterioles and capillaries [1–3], and platelet aggregates were detected in the blood of adult patients during their prolonged TTP episodes. Because all of the samples analyzed were venous blood, and because the extent of vWf binding to the individual platelets within platelet aggregates cannot be evaluated by flow cytometry, it is likely that our observations underestimate the extent of vWf attachment to platelets as they aggregate in the microcirculation of patients.

The plasma vWf multimeric patterns and platelet-bound vWf results in these 9 patients are consistent with the hypothesis that episodes of TTP are associated with the excessive presence of large or unusually large vWf multimeric forms. In CRTTP, which usually begins in childhood, there may be a congenital defect in unusually large vWf processing [30]. The accumulation of released unusually large vWf forms in the circulation of CRTTP patients may periodically exceed a threshold level required for shear-induced intravascular aggregation.

The presence of unusually large vWf forms in the plasma of adult patients with single episode or recurrent TTP may reflect systemic endothelial cell perturbation [31], resulting in the outpouring from endothelial cells of unusually large vWf multimers. It is possible that the outpouring of unusually large vWf forms may overwhelm unusually large vWf processing by a natural plasma unusually large vWf protease [32–34] or other ''reductase'' [35,36] activity. Alternatively, there may be some transient inhibition of unusually large vWf processing [8,37], as by an autoantibody or a chemical or microbial toxin. Our results suggest that disappearance of large plasma vWf forms from some plasma samples of adult patients during acute TTP episodes is at least partially explained by the binding to platelets of vWf multimers, perhaps including unusually large vWf forms.

Fluid shear stresses (i.e., the relative parallel motion between fluid planes during flow) in the microcirculation may be important in inducing the attachment of unusually large vWf multimers, (along with the largest plasma vWf forms), onto vWf receptors, causing aggregation [4,17]. Shear-induced aggregation requires only a small amount of vWf binding to platelet vWf receptors [5,6,19–21,38,39]. Direct platelet aggregation (i.e., aggregation not proceeded by platelet-subendothelial adhesion) may account for the reversible formation of platelet thrombi during TTP episodes in areas of the microcirculation with high-fluid shear stresses, but no endothelial cell desquamation. The initial formation of thrombi in the microcirculation during TTP episodes may increase further shear stresses on platelets in flowing blood and, therefore, potentiate and perpetuate shear-induced platelet aggregation.

Animal studies indicate that the binding of intact vWf multimers of various sizes to the GPIb component of

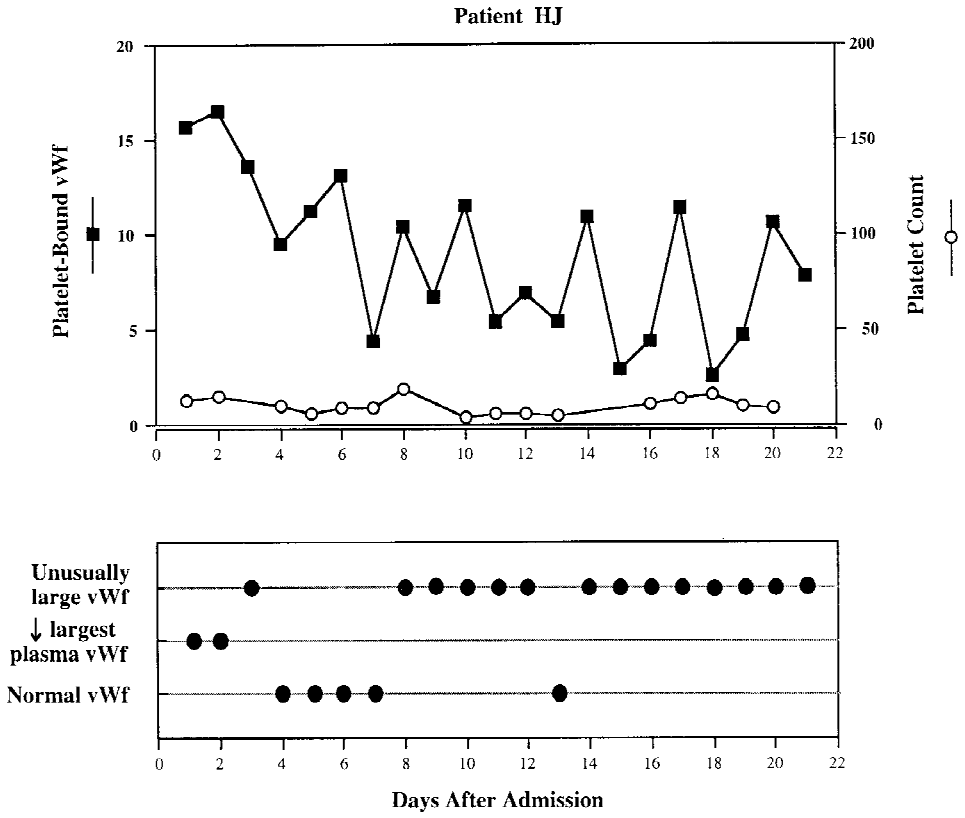

Fig. 7. Platelet counts, vWf on single platelets, and vWf multimeric patterns in patient HJ, who died during her single TTP episode. Splenectomy was performed on day 16.

platelet GPIb-IX-V receptors may be sufficient to initiate episodes of thrombotic thrombocytopenia. The animal models developed so far that most closely approximate thrombotic microangiopathy in humans have utilized the rat, dog, or pig. In each animal, platelet clumping can be induced in vivo by the binding to platelets of plasma vWf multimers that have formed complexes with injected botrocetin [40–42]. Circulating vWf, rather than any vWf released from the α-granules of animal platelets, is required for this phenomenon [42]. The lack of concurrence of vWf and P-selectin expression on single platelets in all samples during acute episodes from 3 of the adult TTP patients may also be the result of binding to platelets of vWf from the plasma of these individuals, rather than release onto their platelet surfaces of the contents of α-granules [43,44].

These direct flow cytometry studies on TTP patient whole blood samples indicate that the binding to platelets of vWf multimeric forms occurs during single and recurrent episodes of TTP. The similar results in the different types of TTP suggest that, regardless of the underlying etiology of the different types of TTP, microvascular platelet aggregation is likely to occur through a common vWf-mediated pathway. It may, therefore, be useful to investigate the possible therapeutic effect of specific blockade of vWf binding to platelets in patients with TTP.

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